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Research Article

Aluminum-Tolerant *Pisolithus* Ectomycorrhizas Confer Increased Growth, Mineral Nutrition, and Metal Tolerance to *Eucalyptus* in Acidic Mine Spoil

Louise Egerton-Warburton[1,2]

[1]*Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60022, USA*
[2]*Program in Plant Biology and Conservation, Weinberg College of Arts and Sciences, Northwestern University, Evanston, IL 60208, USA*

Correspondence should be addressed to Louise Egerton-Warburton; lwarburton@chicagobotanic.org

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Ectomycorrhizal fungi (ECM) may increase the tolerance of their host plants to Al toxicity by immobilizing Al in fungal tissues and/or improving plant mineral nutrition. Although these benefits have been demonstrated in *in vitro* (pure culture) or short-term nutrient solution (hydroponic) experiments, fewer studies have examined these benefits in the field. This study examined the growth, mineral nutrition, and Al levels in two *Eucalyptus* species inoculated with three *Pisolithus* ecotypes that varied in Al tolerance (*in vitro*) and grown in mine spoil in the greenhouse and field. All three ecotypes of *Pisolithus* improved *Eucalyptus* growth and increased host plant tolerance to Al in comparison to noninoculated plants. However, large variations in plant growth and mineral nutrition were detected among the *Pisolithus*-inoculated plants; these differences were largely explained by the functional properties of the *Pisolithus* inoculum. Seedlings inoculated with the most Al-tolerant *Pisolithus* inoculum showed significantly higher levels of N, P, Ca, Mg, and K and lower levels of Al than seedlings inoculated with Al-sensitive ecotypes of *Pisolithus*. These findings indicate an agreement between the fungal tolerance to Al *in vitro* and performance in symbiosis, indicating that both ECM-mediated mineral nutrient acquisition and Al accumulation are important in increasing the host plant Al tolerance.

1. Introduction

Potentially toxic levels of aluminum (Al^{3+}) are a major constraint to plant growth during the restoration of acidic mining soils. Exposure to Al generally results in a severe reduction in growth and productivity due to the inhibition of root cell division and elongation and reductions in the uptake of water and assimilation of nutrients including N, P, Ca, and Mg. Certain plant families show an innate tolerance to acidic soils and Al [1] and some trees species are thought to resist elevated soil metal concentrations by means of a large phenotypic plasticity [2]. For many tree species, however, symbioses with a small guild of well-adapted ectomycorrhizal (ECM) fungi reduce the sensitivity of roots and plants to Al stress [3].

Many trees species form symbiotic associations with ECM fungi. These fungi provide nutrients and water to trees in exchange for carbohydrates and may also play a crucial role for tree regeneration in soils contaminated with metals. *In vitro* studies indicate that ECM fungi may tolerate high Al concentrations [4, 5] and show wide inter- and intraspecific variations in sensitivity to Al and other phytotoxic metals [6, 7]. For example, studies showed that *Laccaria* was more tolerant of high Al availability than *Hebeloma* or *Lactarius* [4, 8], and *Pisolithus* and *Suillus* were more tolerant of Al than *Thelephora* [5]. In addition, tolerance may be correlated with soil metal concentrations at the site of origin [9] but not always [5].

In symbiosis, ECM fungi may show broad ameliorative effects on woody plant responses to acidification [10] and phytotoxic metals, including Al, Cu, Ni, and Zn [6, 11]. As a result, plants inoculated with ECM generally maintain better growth and reduced transfer of metals to shoots than

Table 1: The percentage of soil separates and levels of pH, nutrients, and Al in soil and mining spoil from each of the *Pisolithus* sporocarp collection sites. Values represent the mean with standard error in parentheses. For each column, means with the same letter do not differ significantly at $p < 0.05$.

Site	Sand	Silt	Clay	pH	N	C	P	Al	Mn	Ca	Mg	K
	%				%			$\mu g\,g^{-1}$ soil		Meq $100\,g^{-1}$ soil		
Mine	58	21	21	4.60^{b} (0.06)	0.029^{b} (0.01)	5.71^{b} (1.5)	0.68^{c} (0.23)	320^{a} (45)	631^{a} (105)	0.12	0.51	0.03
Restored	81	6	13	4.79^{b} (0.11)	0.034^{b} (0.01)	10.23^{a} (1.2)	2.66^{a} (0.34)	36^{b} (5)	298^{b} (129)	0.09	0.40	0.01
Forest	73	8	19	5.61^{a} (0.26)	0.131^{a} (0.01)	5.45^{b} (1.3)	1.78^{b} (0.23)	10^{c} (3)	156^{b} (99)	6.00	4.10	0.30

nonmycorrhizal plants when exposed to acidity and/or an excess availability of Al [11]. Such ECM-mediated metal tolerance has been attributed to various extracellular (chelation, cell-wall binding) or intracellular detoxification mechanisms such as binding to nonprotein thiols or vacuolar storage [1, 4, 11–14]. In other studies, however, the alleviation of Al or heavy metal toxicity by ECM was attributed to improved plant mineral nutrition owing to an increase in the uptake of poorly soluble ions (P) or base cations (Ca, Mg) [4, 10, 15, 16]. Further, the effects of ECM on plant Al tolerance may depend on the identity of the host species or ECM-host combination. For example, inoculation with *Laccaria* conferred Al tolerance to both *Fagus* and *Pinus* whereas inoculation with *Paxillus* conferred tolerance only to *Pinus* [10]. In acidic soils, *Xerocomus badius-Picea abies* ECM showed a higher potential to store N, K, Mg, and metals in the fungal sheath than other ECM-*Picea* combinations [12].

Despite this increasing knowledge base, there are still surprisingly few field experiments that test for relevant differences in Al tolerance in ECM-host tree combination [4, 17]. Instead, most of the reported effects of ECM on Al toxicity and plant growth have been generated in short-term nutrient solution (hydroponic) experiments [8], and these results do not always accurately predict plant field performance [18]. This study examined the growth and mineral nutrition of two *Eucalyptus* species inoculated with three *Pisolithus* ecotypes when grown in acid mine spoil in the greenhouse and field. The *Pisolithus* ecotypes were collected from coal mine spoil that differed in soil Al levels and were previously found to show varying levels of adaptive tolerance to Al [9]. *Pisolithus* is a cosmopolitan genus that forms ECM associations with a broad range of angiosperm and gymnosperm tree species, including *Eucalyptus* [19]. It is also a common pioneer on mine sites and increases plant growth in adverse soil conditions [17]. The results were used to address three questions. (1) Do *Pisolithus* isolates from soils with the highest Al levels provide the greatest benefits to seedlings growing in mine spoil? (2) Does the effect of the *Pisolithus* isolates on plant growth vary with host plant identity? (3) If there is an effect of *Pisolithus* isolates on seedling growth, can this be attributed to reduced Al accumulation in plant tissues or improved plant nutrition?

2. Materials and Methods

Seed, inoculum, and soil for the pot trial were all sourced from the lease of Western Collieries Ltd., which is located in the Collie Coal Basin, Western Australia (33°26′S, 116°12′E). The Collie Coal Basin occurs in a significant physiognomic depression of the Darling Plateau in southwestern Australia. The region is typified by a warm Mediterranean-type climate and receives, on average, 993 mm precipitation per annum. Rainfall occurs from May to October (Southern Hemisphere winter-spring) followed by five to six months with little or no rainfall (Southern Hemisphere summer-autumn). Forest soils are typically nutrient poor leached sands (Table 1). In contrast, coal-mining soils are characterized by water repellence and composed of coarse nonbound sands with significant increases in the proportion of silt and clay in comparison to forest soils (Table 1). Mine spoil is also more acidic and contains higher levels of Al and Mn and lower levels of N, P, Ca, Mg, and K than forest soils (Table 1).

2.1. Pisolithus Inoculum Preparation. *Eucalyptus* seedlings were inoculated with cultures of *Pisolithus* previously identified as varying in Al tolerance [9]. Briefly, *Pisolithus* was isolated from sporocarps collected under *Eucalyptus* trees in abandoned mine sites, restored sites, and native forest sites (Table 1) and sequentially cultured to produce pure culture. *In vitro* screening in Al-amended solid media identified variations in Al tolerance among the *Pisolithus* isolates, whereby *Pisolithus* that was isolated from abandoned and restored mine sites was highly tolerant of Al (as Al^{3+}), and *Pisolithus* isolated from an adjacent native forest showed significantly lower tolerance to Al [9]. Intersimple sequence repeat (ISSR) fingerprinting also revealed strong intraspecific variation among these *Pisolithus* isolates (Egerton-Warburton, unpublished data). These isolates were grown in bulk and then encapsulated in hydrogel beads [20] as this comprised an efficient method for the large-scale inoculation of containerized seedlings.

2.2. Greenhouse Trial. Two common forest trees used in restoration, *Eucalyptus rudis* Endl. and *Eucalyptus patens* Benth., were used in this study. Seeds of each species were collected from mature trees growing in abandoned mine sites on the lease of Western Collieries Ltd. at Collie. Half-sibling

progeny was collected from an individual tree to reduce the effect of genetic variation within the seed stock on the trial. Seeds were surface sterilized with 3% v/v $NaHClO_4$ for 5 min, washed in three changes of sterile deionized water, and then transferred to moistened sterile filter paper in petri dishes. Seeds were incubated in darkness at room temperature (20°C) for eight days before transplanting into prepared pots of mine spoil.

Mine spoil was used as the growing medium. These materials were classified as a sandy loam (73.8% sand; 16.2% clay; 10.1% silt), acidic (pH 4.46 ± 0.4, mean ± s.e.), with high levels of Al (327 ± 45 $\mu g\, g^{-1}$ soil) and low levels of plant-available P (0.24 ± 0.02 $\mu g\, g^{-1}$ soil) and available moisture (6.8%–11%). Soils were sieved to 2 mm and then 1.5 kg was placed in each of 240 plastic pots lined with polythene bags that had been perforated at the base to allow drainage. Forty replicate pots per inoculum source plus 40 replicate pots of noninoculated pots (control) were established for each *Eucalyptus* species. For each species and inoculum source, four germinants were placed in an individual pot and supplied with eight hydrogel beads (two per plant) in the root zone. After planting, N and P fertilizers were applied once in solution to the soil surface at rates of 2 mg P kg^{-1} soil (as KH_2PO_4) and 2 mg N kg^{-1} soil (as NH_4NO_3). No further fertilizers were applied during the trial. Soils were watered to 8% soil moisture content and maintained in a greenhouse (30°C max, 21°C min). Pots were segregated by inoculum source to reduce the possibility of cross-contamination. At the four-leaf stage, seedlings were thinned to one per pot and any cotyledons were removed from seedlings to promote mycorrhization. Soil moisture levels were maintained by bringing pots to field capacity twice a week.

Seedlings were destructively harvested after 150 days of growth. At harvest, plants were washed gently from pots and divided into roots and shoots. Root material was subdivided into coarse and fine roots, with subsamples of fine roots from each treatment and species inspected for the abundance, color, and morphology of the colonising mycorrhizas. Short roots with fully developed sulfur yellow mantles and emanating hyphae were considered mycorrhizas of *Pisolithus*. Mycorrhizal colonization was expressed as the percentage of lateral fine roots colonized. The remaining root and shoot materials were dried to constant weight at 60°C and dry weights recorded. Subsamples of shoot material of each *Eucalyptus* and inoculum treatment were then ground to a fine powder and analyzed for N, P, K, Ca, Mg, and Al by CSBP and Farmers Analytical Laboratory (Bayswater, Western Australia; http://www.csbp-fertilisers.com.au/csbp-lab).

2.3. Field Trial. Seedlings of *E. rudis* and *E. patens* were inoculated with mine or forest site isolates of *Pisolithus* and assessed for growth and mycorrhization in the field. Seedlings inoculated with *Pisolithus* from the restored sites were not included in this comparison since the growth responses and nutrient levels were largely similar to those in seedlings grown with mine site inoculum (see Figure 1). In addition, noninoculated plants were not included in this assessment as their survival was extremely poor (<15%) in pot trials. Seeds were germinated as described previously. For each species and inoculum source, a single germinant was placed in an individual Jiffy pots (capacity 50 mL) containing sieved Collie topsoil, which is classed as sand (93% sand; 2.4% clay; 4.6% silt) with pH 5.3, extractable Al 2 $\mu g\, g^{-1}$ soil, and P 1.6 $\mu g\, g^{-1}$ soil. Seedlings were supplied with hydrogel bead (two per plant) in the root zone. Supplemental fertilization of forest soil was not required owing to the higher levels of plant-available P. Sixty-four seedlings of each species and inoculum type were established (total = 256 plants). Seedlings were maintained in a shade house and watered daily with an automatic watering system for four months prior to planting.

Seedlings were planted into a prepared rehabilitation site on the lease of Western Collieries Ltd. at Collie. The site had been prepared by battering the subsoil to a 9° slope followed by a dressing of local forest topsoil and a fertilizer application of 10 kg P ha^{-1} as single superphosphate (9% P). Eighteen months after planting, the surviving seedlings were excavated from the field site and processed for mycorrhizal colonization, root and shoot biomass, and mineral nutrient content as described previously (see Section 2.2 Greenhouse Trial).

2.4. Data Analyses. The differences in total plant biomass (root, shoot) and foliar mineral nutrient levels (pot, field trials) and mycorrhizal colonization (pot trial) were analyzed using analyzed linear mixed models with restricted maximum likelihood (REML) estimates. In the analyses, *Eucalyptus* host species and *Pisolithus* inoculation source (mine, restored, and forest) were specified as random effects. Because both host plant and mycorrhizal partner can have large influences on host nutrition, the relative contribution of *Eucalyptus* host species and *Pisolithus* inoculum source on plant mineral nutrition was tested using REML linear models. Biomass accumulation was modeled as a function of the fixed effect of plant nutrient levels (N, P, K, Ca, Mg, and Al), with *Eucalyptus* host and *Pisolithus* source specified as random effects. To determine the combinations of nutrients with the greatest explanatory power, each variable (mineral nutrient) was added into the model in a stepwise fashion. Briefly, variables that were found to be significant in the presence of previously fitted variables were retained in the model, while variables that were no longer significant in the presence of other variables were removed. The final combined models were used to report the variance explained by plant host and inoculum source in the pot and field trials. In addition, variables retained in the model were tested using *post hoc* multiple *t*-tests to determine significant differences in mineral nutrients between plant hosts or inoculum sources. All variables were tested for normality and, where necessary, log transformations were applied prior to analysis.

3. Results

3.1. Greenhouse Trial. *Eucalyptus* host ($p < 0.001$) influenced plant root and shoot biomass accumulation whereby *E. rudis* plants were significantly larger than *E. patens* plants (Figure 1(b)). Shoot biomass in *E. patens* and *E. rudis* was significantly higher ($p < 0.001$; Figure 1(a)) in inoculated than

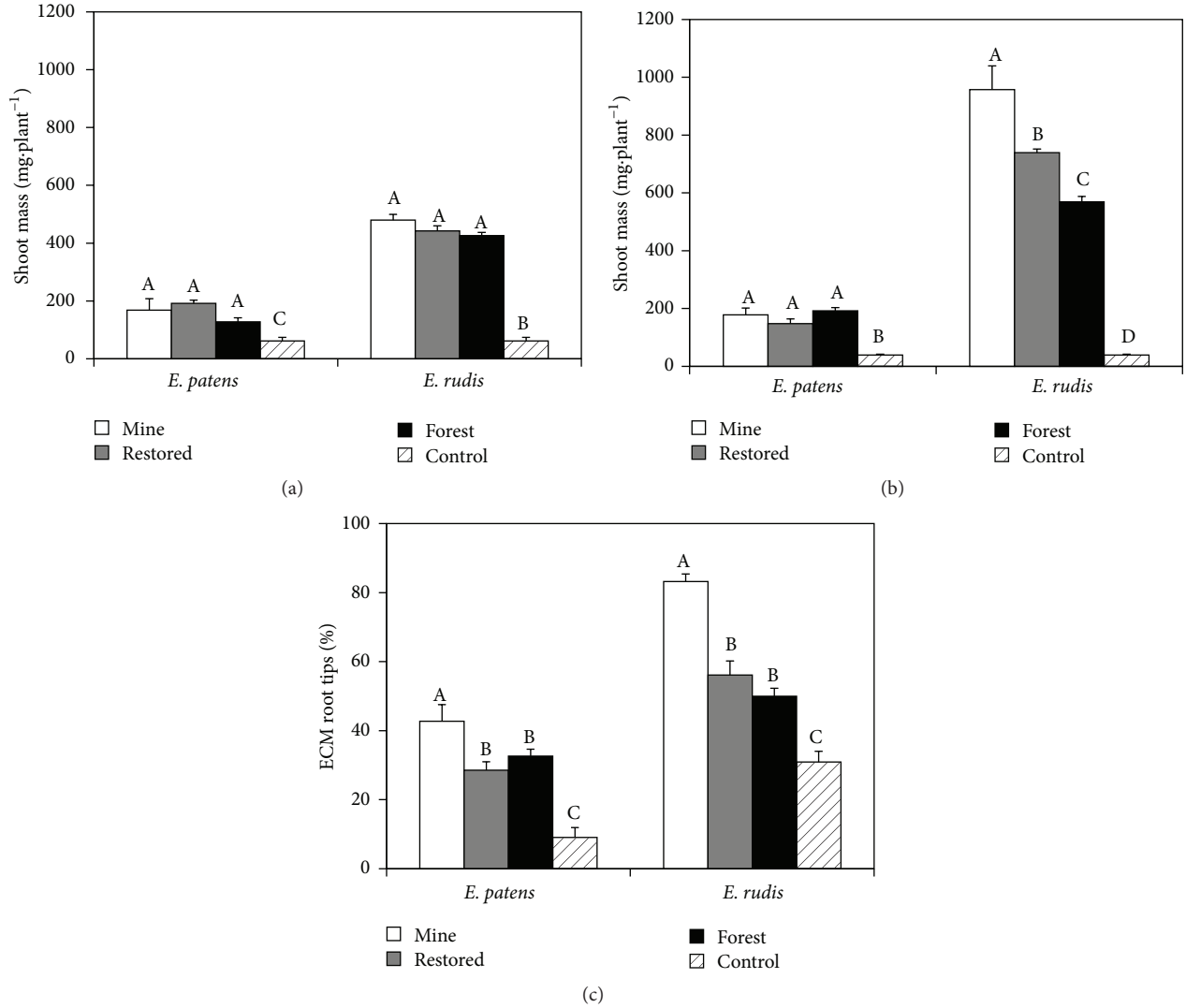

Figure 1: Effect of mine, restored, or forest site *Pisolithus* ecotype on the shoot biomass (a), root biomass (b), and fine root ectomycorrhizal colonization (c) in *Eucalyptus* plants in the pot trial. Vertical bars indicate the standard error of the mean. For each plant species, mean biomass or root colonization values with the same letter do not differ significantly at $p < 0.05$.

in noninoculated (control) plants, but there was no difference in shoot biomass among *Pisolithus* sources ($p = 0.274$). Root biomass in *E. patens* and *E. rudis* was also significantly higher ($p < 0.001$; Figure 1(b)) in inoculated than in noninoculated (control) plants. In *E. patens*, there was no significant difference in root biomass among *Pisolithus* sources ($p = 0.267$) whereas *E. rudis* inoculated with *Pisolithus* from mine sites produced significantly greater root biomass than when inoculated with *Pisolithus* from restored or forest sites ($p = 0.002$).

Both *Eucalyptus* host ($p < 0.001$) and *Pisolithus* source ($p < 0.001$) influenced mycorrhizal root colonization (Figure 1(c)). Root colonization was significantly higher in *E. rudis* (54 ± 6%; mean ± s.e.) than *E. patens* (26 ± 3%). In addition, 61 ± 9% root tips were colonized in plants inoculated with mine site *Pisolithus* compared with 41 ± 6% for restored site and 43 ± 5% for forest site *Pisolithus*. There was no significant *Eucalyptus x Pisolithus* source interaction ($p = 0.401$).

Results from REML (Table 2) revealed that variations in plant N, P, and Ca (positive) and Al content (negative) significantly influenced biomass accumulation, and such variations were largely explained (68.4%) by the functional properties of the *Pisolithus* inoculum. Seedlings inoculated with mine or restored site *Pisolithus* inoculum showed significantly higher levels of N, P, and Ca, and lower levels of Al than seedlings inoculated with forest site *Pisolithus* ($p < 0.001$; Figures 2(a), 2(b), 2(d), and 2(f)). Noninoculated seedlings contained the lowest levels of mineral nutrients and highest levels of Al. Levels of K and Mg were also significantly higher in seedlings inoculated with mine or restored site than forest site *Pisolithus* ($p < 0.001$; Figures 2(c) and 2(e), resp.), but these nutrients were not significant in REML models of biomass accumulation.

Table 2: Residual maximum likelihood (REML) results of the influence of *Eucalyptus* species and *Pisolithus* isolate source as explanatory factors on plant biomass and mineral nutrition in pot and field trials. The significant fixed effects (nutrients), their standardized estimates with standard error (s.e.), p values, and the amount of variation explained by plant and mycorrhizal parameters are presented.

Response variable	Retained fixed effect	Estimate (s.e.)	p	*Eucalyptus* species (%)	*Pisolithus* source (%)
Biomass (pot)	N	0.164 (0.09)	0.004	26.9	68.4
	P	1.075 (0.03)	<0.001		
	Ca	0.858 (0.22)	0.010		
	Al	−0.518 (0.07)	0.008		
Biomass (field)	N	0.726 (0.08)	0.004	27.1	58.6
	P	1.990 (0.21)	0.007		
	Ca	0.226 (0.02)	0.005		
	Mg	0.275 (0.06)	0.008		
	Al	−0.477 (0.04)	0.002		

3.2. Field Trial. *Eucalyptus* host ($p = 0.003$) and *Pisolithus* source ($p = 0.006$) influenced plant biomass accumulation after 18 months in the field (Figure 3(a)). There was no significant *Eucalyptus x Pisolithus* source interaction ($p = 0.513$). *Eucalyptus rudis* was significantly larger than *E. patens*, and *Pisolithus* from mine sites resulted in greater plant biomass than *Pisolithus* from the forest site.

Overall levels of ECM root colonization did not differ significantly between seedlings receiving mine site (74 ± 4%; mean ± s.e.) or forest sources of *Pisolithus* (77 ± 3%). However, five different morphological types of ECM, including *Pisolithus*, colonized root tips. *Pisolithus* was readily identified by the presence of a coarse, sulfur yellow mantle, emanating hyphae, and rhizomorphs. One ECM was consistent with *Cenococcum* (black mantle, short black hyphae), while the other was an unknown type characterized by a deep brown mantle and short radiating external hyphae. In fact, after 18 months of field growth, *Pisolithus* mycorrhizas comprised, on average, only 19 ± 4% of ECM root tips in seedlings receiving mine site inoculum and 7 ± 3% of ECM root tips in seedlings receiving forest inoculum.

Results from REML analyses for field-grown plants were similar to those from the pot trial: variations in plant N, P, Ca, and Mg (positive) and Al (negative) influenced plant biomass accumulation (Table 2). In addition, these variations were better explained by the properties of the *Pisolithus* inoculum (58.6%) than host plant. Plant N levels were significantly higher in seedlings inoculated with mine (1199 ± 18 mg N plant^{-1}; mean ± s.e.) than forest site *Pisolithus* (1176 ± 7 mg N plant^{-1}; $p = 0.018$). In addition, levels of plant K ($p < 0.001$), Ca ($p < 0.001$), Mg ($p < 0.001$), and P ($p = 0.009$) were significantly higher, and Al levels were significantly lower ($p < 0.001$) in plants inoculated with mine than forest site *Pisolithus* (Figure 3(b)).

4. Discussion

An extensive literature has shown that ECM fungi from metal-contaminated soils may present a higher metal tolerance (*in vitro*) than isolates from noncontaminated soils and that intraspecific variation in metal tolerance can occur among isolates [3, 6, 7, 9]. However, questions have remained about whether the adaptive metal tolerance observed *in vitro* might result in increased protection against metal toxicity in a host plant growing in contaminated soils. In this study, all three ecotypes of *Pisolithus* improved *Eucalyptus* growth and mineral nutrition (N, P, Ca, Mg, and K) and reduced the transfer to a phytotoxic metal (Al) to the host plant in comparison to nonmycorrhizal seedlings. These trends are in general agreement with earlier studies of ECM plants in metal-contaminated and native soils [3, 11, 15]. More notably, the experiments revealed that the source of the *Pisolithus* isolate was the largest influence of plant growth, mineral nutrition, and Al content.

Overall, *Eucalyptus* seedlings inoculated with mine *Pisolithus* produced the largest growth responses and forest *Pisolithus* the least. The magnitude of this effect is most obvious in *E. rudis* (pot trial) and in shoot biomass accumulation in the field trial (both *Eucalyptus* species). Because the study used half-sib progeny and confined plant growth to a single substrate (mine spoil), these results suggest that genetic differences existed between *Pisolithus* isolates in their ability to benefit seedling growth [3, 6, 21]. In addition, plant growth responses were largely consistent with the patterns of *Pisolithus* Al tolerance reported in *in vitro* experiments [9], suggesting a reasonable agreement between the fungal sensitivity to specific metals *in vitro* and performance in symbiosis in acidic mine spoil (Question 1).

Even so, there were large variations in the symbiotic and physiological effectiveness of *Pisolithus* between host species: *E. rudis* showed high levels of mycorrhizal root colonization and correspondingly larger growth responses to *Pisolithus* in comparison to *E. patens* (Question 2). This result is not surprising given the wide range of physiological responses that have been reported in host species inoculated with *Pisolithus* [22, 23]. What was surprising, however, was that all three isolates of *Pisolithus* produced similar root and shoot growth responses in *E. patens* (pot trial). While it is possible, this result represents some form of fungal-host incompatibility between *Pisolithus* and *E. patens* [21, 23], the more likely explanation is that *Pisolithus* acted as a strong C sink during the early stages of the symbiosis [24]. *Eucalyptus patens* grown with mine site *Pisolithus* for 4 months (pot trial) showed the highest levels of root colonization but limited

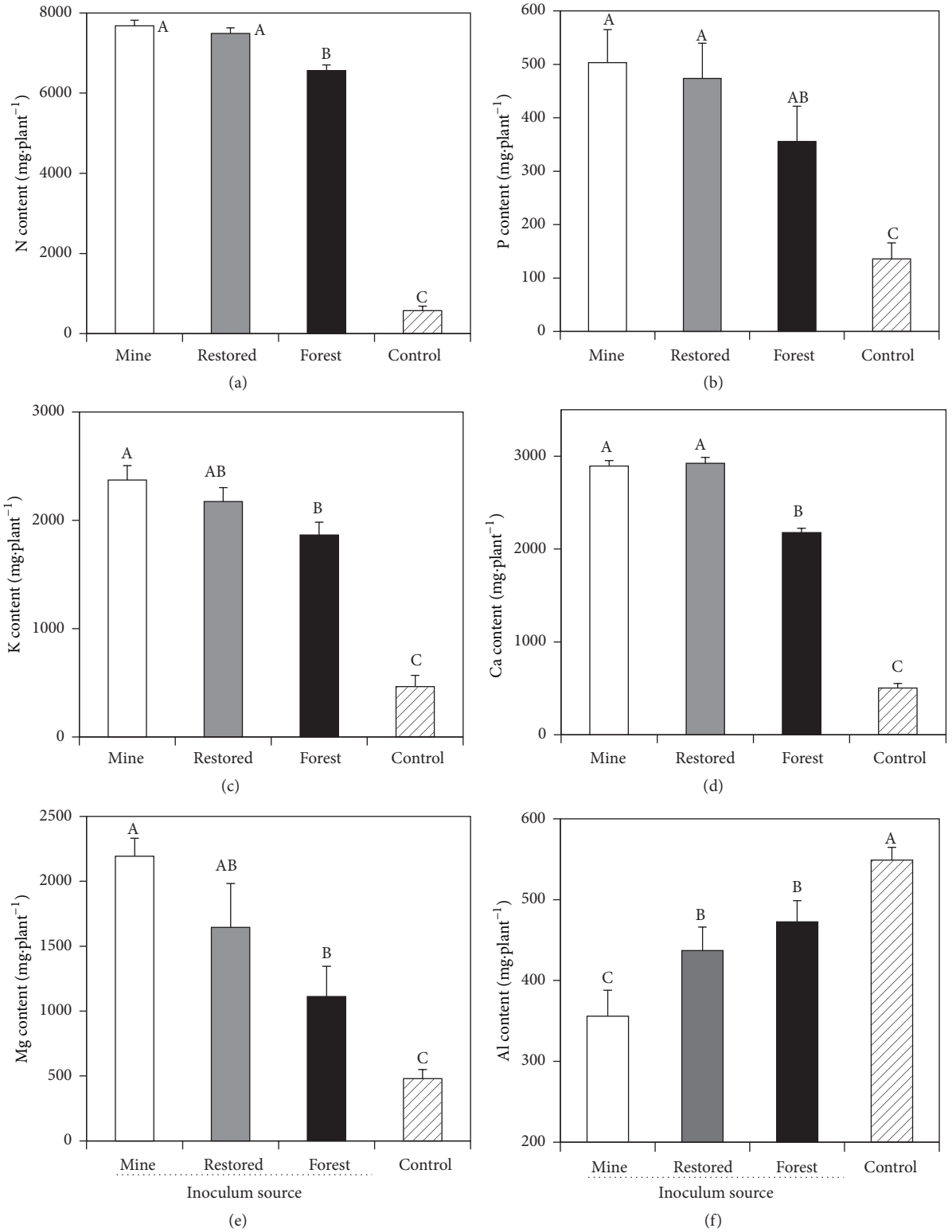

Figure 2: Effect of inoculation with mine, restored, or forest site *Pisolithus* ecotype on the mean foliar N (a), P (b), K (c), Ca (d), Mg (e), and Al levels (f) in *Eucalyptus* plants in the pot trial. Vertical bars indicate the standard error of the mean. For each nutrient, mean levels with the same letter do not differ significantly at $p < 0.05$.

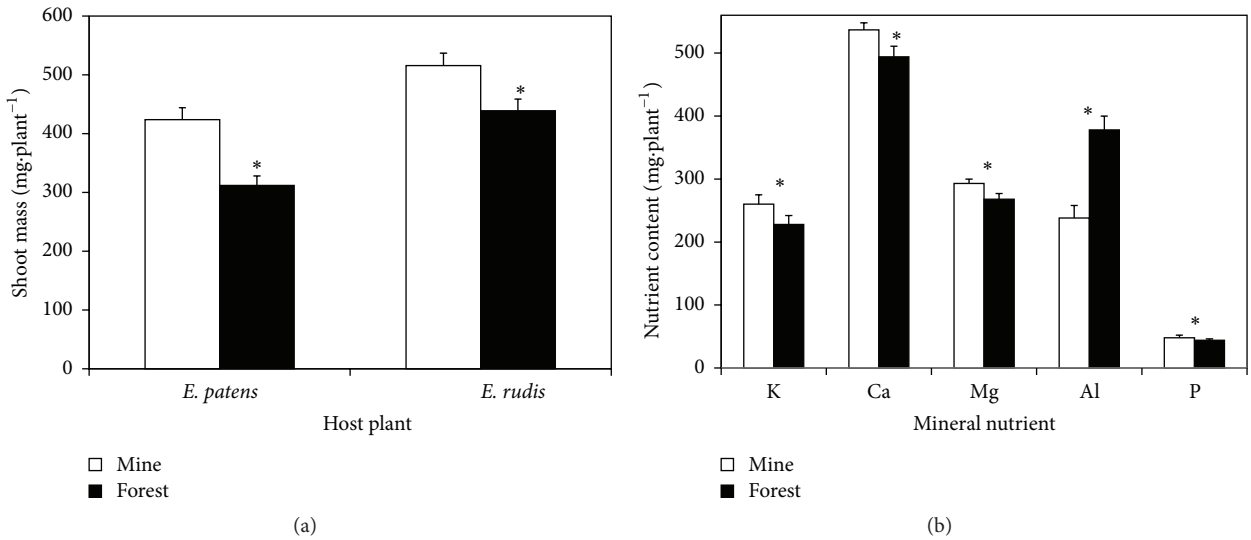

FIGURE 3: Effect of inoculation with mine or forest site *Pisolithus* ecotype on shoot biomass (a) and foliar levels of K, Ca, Mg, Al, and P (b) in *Eucalyptus* plants in the field trial. Vertical bars indicate the standard error of the mean. For each plant species (a) or nutrient (b), mean values indicated with an asterisk (*) differ significantly at $p < 0.05$; ns: not significantly different ($p > 0.05$).

biomass accumulation. After 18 months of field growth, however, root colonization and seedling biomass in *E. patens* were similar to *E. rudis*. These results support the idea that early symbiosis may have acted as a temporary C sink in *E. patens*.

At least two general types of mechanisms apparently contributed to the enhanced growth response in *E. rudis* with mine site *Pisolithus* (Question 3). The most obvious was the ability of the ECM partner to reduce Al levels within the plant. In plants grown with mine site *Pisolithus*, Al levels remained low in comparison to plants grown with forest *Pisolithus*. These experiments were not intended to evaluate the specific mechanisms by which ECM fungi might alleviate Al toxicity. However, the significant differences in foliar Al levels between nonmycorrhizal and *Pisolithus*-inoculated plants indicate that mycorrhizal structures may have limited Al penetration into the root symplasm and subsequent transport to the shoot [11, 25]. Binding of Al to the fungal cell walls represented a substantial fraction of the metal accumulated by *Pisolithus* mycorrhizas on *Eucalyptus* [9] and *Pinus* [26] and may well be part of the mechanism by which ECM *Eucalyptus* tolerated the high levels of the metal in spoil in the current study. In addition, studies with *Pisolithus* and other ECM fungi have highlighted a role for Al detoxification within the fungal vacuole with S-rich substrates or P-rich granules [12–14, 27] and the capacity of *Pisolithus* mycelia to produce low molecular-weight organic acids that bind Al and prevent its absorption [4, 25, 28]. Although it is uncertain which processes might be involved, the reduction in Al levels in ECM *Eucalyptus* in this study implies an efficient and substantial tolerance mechanism.

A second type of tolerance mechanism appeared to be the maintenance of nutrient acquisition. Mycorrhizal *E. rudis* contained significantly higher levels of N, P, K, Ca, and Mg than nonmycorrhizal plants in mine spoil. This is particularly important because mine spoil is largely deficient in essential plant nutrients such as N, P, and K, and high levels of Al are known to limit the uptake of divalent cations (Ca, Mg). Indeed, the stunted root growth and low levels of foliar N, P, K, Ca, and Mg in nonmycorrhizal *E. rudis* and *E. patens* were consistent with Al-induced growth impairments in woody plants [25] and competitive inhibition of Mg and Ca uptake [16, 29]. In contrast, plants grown with *Pisolithus* showed substantial increases in N and P, consistent with the physiological role of ECM in natural forest or Al-treated soils [4, 10, 25], as well as increased plant Mg, Ca, and K levels. This result suggests that an ECM-mediated uptake of cations might directly ameliorate the effects of Al [4, 8, 16] in a manner similar to other phytotoxic metals [11, 15], or indirectly by enhancing P uptake [30]. Taken together with plant Al levels, these findings suggest that both plant nutrient imbalances and direct Al toxicity are likely the main reasons for the negative effects of mine spoil on plant growth.

It is interesting to note that inoculation with mine site *Pisolithus* resulted in the greatest increases in plant growth and nutrient status, and forest *Pisolithus* resulted in the smallest increases. This outcome agrees with earlier findings showing the high intraspecific variability of *Pisolithus* (and other ECM fungi) to promote the mineral nutrition of the host plant [21, 22, 31]. However, other key factor(s) besides nutrient acquisition may be involved. *Pisolithus* produces long distance exploration hyphae with hydrophobic cell walls that are capable of transporting physiologically significant quantities of water through the soil-plant continuum [32]. This is notable because mine spoil shows low water holding capacity, and juvenile *Eucalyptus* growth is strongly and negatively influenced by soil moisture deficits. The inoculation of woody plants with *Pisolithus* has been shown to mitigate the effects of drought by improving water acquisition or modifying plant ecophysiological responses to soil moisture deficits [31]. Because genotypes of *Pisolithus* differ markedly in their capacity to confer drought tolerance to their host [31],

it is tempting to speculate that *Pisolithus*, and especially the mine site isolate, may have enhanced the growth of *E. rudis* by ameliorating the effects of soil moisture deficits. Given the potential importance of this mechanism, further field-based experimentation is needed to determine the impact of the different *Pisolithus* isolates on *Eucalyptus* ecophysiology.

5. Conclusions

Inoculation with *Pisolithus* appeared to compensate for inefficient plant nutrient acquisition as well as bestow increased Al resistance in a host plant that showed a level of sensitivity to spoil conditions. *Pisolithus* isolates also differed markedly in their capacity to confer such benefits to their host indicating that screening and selecting fungal isolates based on their capacity to adapt to abiotic stresses in the spoil, such as Al, may be a prerequisite to using ECM isolates for larger scale inoculation trials or restoration. Although the potential exists to utilize the most Al-tolerant ECM, it was also clear that not all *Eucalyptus* species would benefit from inoculation during early seedling establishment. As a result, screening the performance of various *Pisolithus-Eucalyptus* species combinations in mine spoil comprises a logical second step in planning for future field planting.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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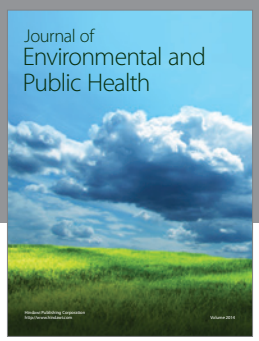

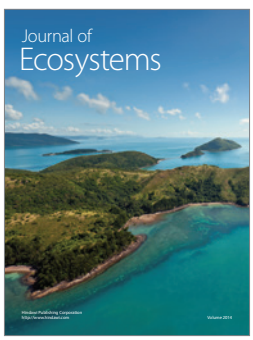

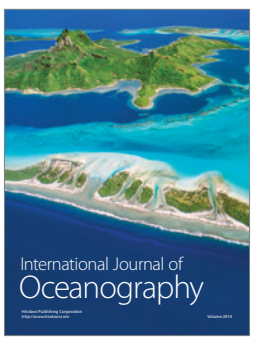

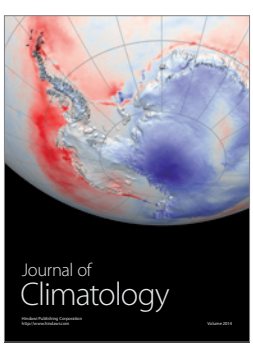

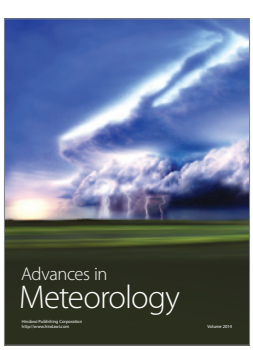

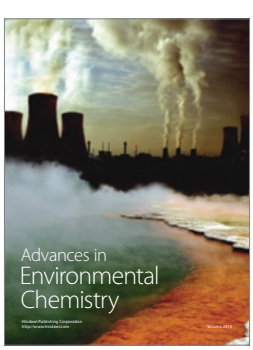

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